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TOBACCO SPECIFIC NITROSAMINE LEVELS OF MARAS POWDER (TURKISH SMOKELESS TOBACCO)

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Keywords:
Maras powder, nitrosamines.

Abstract

Objective: One of the preparations of smokeless tobacco different from other branded and locally produced ones in Turkey is Maras powder (Mp). Mp contains carcinogenic tobacco-specific nitrosamines (TSNA) which are common among smokeless tobacco products. There is no specific research analyzing nitrosamine levels in Mp. In the present study, we aim to investigate the levels of nitrosamine of Mp.

Method: N' nitrosonornicotine (NNN), 4-(nitrosometil-amino)-1-(3-pyridil)-butanon (NNK), N' Nitrosoanatabine (NAT), N' Nitrosoanabasine (NAB), and total TSNA were evaluated in six different Mp mixtures and then analyzed with chromatographic method.

Results: The levels of NNN, NNK, NAT, NAB, and total TSNA were 2.5 ± 0.3 µg/g, 0.7 ± 0.07 µg/g, 1.0 ± 0.1 µg/g, 0.3 ± 0.03 µg/g, and 4.5 ± 0.5 µg/g, respectively. The level of TSNA in wood ash was lower than 0.2 µg/g, which was considered an insignificant level for them. When the levels of nitrosamines in Mp were compared with other smokeless tobacco, they were found to be lower.

Conclusion: These results indicated that non-commercial and impure Nicotiana rustica (NRL) contains lower amounts of nitrosamines that are potentially harmful to humans.

Introduction

Tobacco is used in various ways in many countries [1-6] and Maraş powder (Mp) (Turkish smokeless powder) is one of them. Mp is widely consumed in the southeastern of Turkey especially in Kahramanmaras Gaziantep, Adiyaman, and Sanliurfa. It is produced by drying off the leaves of Nicotiana rustica L. (NRL), which is an open pollinated plant. NRL contains four tobacco-specific alkaloids: 4-(nitrosomethyl-amino)-1-(3-pyridil)-butanon (NNK), N' nitrosonornicotine (NNN), N' Nitrosoanabasine (NAB), and nitrosoanatabin (NAT). NNN, NNK, and NAT are the most abundant and carcinogenic alkaloids [7-8]. Goodsell explain that nicotine is a powerful alkaloid toxin. Nitrosamines are dark face of nicotine; leading directly to a high risk of cancer. Nitrosamines are nicotine-derived compounds, also found in tobacco, which activated within the body to form powerful alkylating agents that attack DNA. Nicotine-derived nitrosaminoketone (NNK) has a reactive nitrosamino group, with a methyl group on one side and larger ketone group on the other. When the activated in the body, either the methyl or ketone group may be transferred to DNA base. This can lead misreading of the genetic information when the DNA is replicated.

The methyl groups from NNK commonly lead to mutations that change a guanine to an adenine [9].
Mp is consumed as a 1 or 2 gram mixture wrapped in cigarette paper and placed into the mouth between the lower teeth and lip for about 5–10 min. This is repeated 5 to 15 times per a day. It is assumed that daily consumption of Mp is around 10-20 g [4]. MP is composed of dry matter of tobacco mixed with wood ash from either oak or grapevine at a ratio of 1:2. It is sold in 10-gram bags at local stores without any commercial name, which means this product has an uncontrolled market. Similar tobacco use is also present in Sudan, to where Mp may have been transported from Anatolia or Saudi Arabia during Ottoman Empire period. It is assumed that the total absorbed tobacco-specific nitrosamines (TSNA) amount from daily use is 13.5 µg/day [4]. Some studies have reported that TSNA may have "pre-cancerous" effects on the tongue, lip mucosa, and gums, and may give rise to pharynx, larynx and gastrointestinal cancers, and oxidative events [6,10-12]. To our knowledge, there has been no research done to measure the level of TSNA in tobacco products grown in Turkey. This work aimed to compare the nitrosamine levels of Mp to those of other smokeless tobaccos in different countries.

Material And Methods

Tobacco Samples

Smokeless tobacco samples locally produced Mp and oak or grapevine wood ash were individually collected from various grocery stores, buffets at six different places in Kahramanmaras and mixed among them. Individual mixtures of MP and wood ash were sent to National Food Administration in Sweden for nitrosamine analysis.

Materials

Nitrosamine standards from Midwest Research Institute (Kansas city, MO, USA) was used and their purity was >95 %. Stock solutions in dichloromethane, pesticide-grade (Lab-Scan, Dublin, Ireland) and working solutions prepared daily in methanol, gradient grade (Merck, Darmstadt, Germany). All solutions were kept in the dark at 2-10 oC.

Apparatus

A Water Alliance 2690 system with a quaternary gradient pump and vacum degassing was used for LC. Separations were carried out using a Genesis C 18 column, 100 mm x 3 mm I.D., 4 µm particle size (Jones Chromatography, Mid Glamorgen, UK) with a 1-cm guard column with the same packing material. The mobile phase was filtered through a 0.45 µm PTFE membrane filter (HVLP, Millipore, Ireland). Separation was performed using a gradient between methanol (mobile phase A) and 10 mM ammonium format, pH 4, in water-methanol (80:20 v/v) (mobile phase B). The gradient was: t = 0 min, 0% A and 100 %B; t = 5 min, 30 % A and 70 % B; 10 min, 30 % A and 70% B; t=12 min, 0% A and 100 % B, next injection volume was 5 µL.

The nitrogen used for desolvation and as nebulising gas for the LC-MS was produced in situ by a nitrogen generator (Aquilo NG 11, Aquilo gas separation Etten-Leur, The Netherlands) fed by compressed air at 7 bar. LS-MS was carried out using a Micromass Quattro LC (Manchester, UK) triple quadrupole mass spectrometer equipped with a standard pneumatically assisted electrospray ion source, operated in the positive ion mode.

Tobacco-specific nitrosamine analyses

The analysis of MP and wood ash analysis was done by Christer Jansson's laboratory in Sweden. As they mentioned that their article [14] first step is extraction for the moist snuff samples, second step analysis of extract on LC-MSMS. Quantification of the unknown snuff samples was calculated by using matrix matched standarts peak high.

Results

Dry matters from Mp and wood ash (oak or grapevine) were divided into two parts and analyzed for TSNA content. The values are shown in Table 1. The highest nitrosamine level among the TSNA levels was NNN and the level of NNN is a major determinant of smoke levels. The level of TSNA was under the 0.1 µg/g DM in wood ash.
TSNAs in smokeless tobacco from different countries are shown in Table 2, and the levels of total TSNA in tobacco cigarette and Turkish Mp are shown in Figure 1. Turkish Mp contains lower amounts of TSNA compared to other commercial smokeless tobaccos, except brand of Wintergreen.

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Discussion

The seeds of NRL used for tobacco cultivation are mostly harvested from the previous year's crops in the region. This procedure causes degeneration of some plant features due to the heterozygote character of the seeds during pollination from both tobacco and other species around the plot. Moreover, local growers in these areas illegally carry out tobacco cultivation. Although the intensive use of Mp is regionally limited in certain areas, the major consumers are mostly from a low socio-economic status, due to the ubiquitous availability and low price of the product. However, there is no specific information available on how often it is used. When the tobacco is left on the shelf for about 6 months, the level of TSNA may increase as much as two-fold [1]. The results of the present study demonstrate that TSNA levels in Turkish MP are relatively low and are comparable with some other conventional brands.

The smoking was forbidden in closed area since 16 th May 2008 by the government. We are thinking that this decision can be increased smokeless tobacco usage among smoking persons. The use of smokeless tobacco varies around the world [2,6,11-14]. This might be due to the different socio-economical and cultural structures of different countries. Mp may be a good example for the extra-ordinary usage of tobacco, and there is no certain evidence for the time Mp was first introduced to our country. It is assumed to have been brought from Europe, and then transported to Middle East and Northern Africa. Despite the fact that cultivation of tobacco in Turkey is under the control of Turkish Government, Mp has been illegally cultivated in small plots by growers and sold in local markets without commercial brand. The growers do not use bred lines and obtain the seeds from the previous year's crop. Tobacco plants have open-pollination features and receive pollen from other crops in their environment. This causes degeneration and loss of some characters in NRL species. There is no research that has measured the nicotine levels in Mp. However, it contains similar amounts of TSNA when compared to other commercial smokeless tobaccos (see, Table 2) such as US snuff tobacco type A and Hawken Wintergreen [1]. Kilinc et al. have reported the similarity of Mp to Tombak in Sudan and also found differences in their TSNA levels [15]. Low levels of TSNA in Mp might be due to open pollination of the plants and use of inbred lines for cultivation by local growers. This can be beneficial for consumers when the harmful effect of high TSNA on human health is taken into account. Use of cigarettes, especially American brands and smokeless tobacco, is thought to be very high, though there is no statistical data on a regional basis in Turkey. Stepanov et al. reported that Turkish tobaccos were used for the production of Moldovian local cigarettes and also that the level of TSNA in Moldavian cigarettes was lower than that of American tobaccos of high quality [13].Harmful effects of both types of tobacco on human health have been reported in many studies [3,7,9,16]. Smoking cigarettes can damage the lungs and bronchial tubes, and smokeless tobacco has carcinogenic effects on mucosa in the mouth, tongue, gums, esophagus, and pancreas [3,11-17] . Since Mp is also another kind of smokeless tobacco, the incidence of pre-carcinogenic lesions, occurrence of leucoplaki or keratosis and also cancer risk can be 50 times more than in a person not using tobacco, due to direct contact of Mp with mucosa, depending upon the duration in the mouth, as it is a different kind of smokeless tobacco [18-21]. However, some authors have proposed that the use of smokeless tobacco (ST) might be less dangerous than smoking cigarettes [18].

Table 2 shows the comparison of nitrosamine levels in some tobaccos grown in different regions. The harmful effect of ST is probably due to the content, amount, daily use and duration of TSNA. Stepanov et al. measured and compared TSNA levels in smokeless tobaccos in India [21] and found that some tobaccos had high NNN and NNK values around 38,9 µg/g and 8.99 µg/g and some of them had 2,24 and 0.71, respectively. Moreover, TSNA levels have not been determined in some groups which were assumed as being locally grown Nicotiana species and lost some features due to open pollination. Mean values from medium to low level in the group were found to be similar to our results. Nilsson indicated that Hoffman et al. reported similarity of the levels of NNN and NNK for snuff dipping (Hawken Wintergreen brand) [22] and Mp. The study of Idris et al. in Sudan showed high TSNA levels in Toombak and also similar use to Mp [20]. The mixture of Toombak and sodium bicarbonate and/or that of MP and wood ash increase alkali levels, which increases absorption. However, there was no significant TSNA content in the wood ash. Regarding TSNA levels absorbed through MP use, the Health Department of the Commonwealth (Massachusetts, USA) proposed to set an upper limit for total TSNA in snuff [1]. While the level of TSNA was found to be very low in smokeless tobacco in many countries (Table 2.), the mean value of TSNA levels of three American commercial tobacco brands was higher than the amount of MP [13], although this value is quite low the differential absorption of TSNA by MP use has to be taken into consideration. It has been reported that absorption

from ST is twice as high as from smoking tobacco [13,21].

Conclusion

In spite of ST contains less nitrosamine and is less detrimental to human health than other kinds of tobacco, it is still dangerous for health and this study must be expanded by additional epidemiological work.

Conflict Of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Table 1: Tobacco Specific Nitrosamines in Turkish Mp and Wood Ash.

		TSNA (µg/g in DM)				
Products	**DM (%)**	**NNN**	**NNK**	**NAT**	**NAB**	**Total TSNA**
Turkish Mp	92.5	2.5 ± 0.3	0.7 ± 0.07	1.0 ± 0.1	0.3 ± 0.03	4.5 ± 0.5
Wood Ash	99.4	<0.02	<0.02	<0.02	<0.02	<0.1

Abbreviations: DM: Dry Matter, Mp: Maras Powder, NNN: nitrosonornicotine, NNK: 4-(nitrosometil-amino)-1-(3-pyridil)-butanon, NAT: N' Nitrosoanatabine, NAB: N' Nitrosoanabasine, total TSNA: Tobacco specific nitrosamines

Table 2: Tobacco specific nitrosamines in smokeless tobaccos in different countries.

		TSNA (µg/g in DM)				
Products		**NNN**	**NNK**	**NAT**	**NAB**	**Total TSNA**
Turkish Mp		2.5±0.3	0.7±0.07	1.0±0.1	0.3±0.03	4.5±0.5
Indian* ST	High levels	38,9*±27.0	8.99±13.0	-	-	-
	Medium to low	2,24±2.63	0.71±0.86	-	-	-
US oral snuff** tobacco	Type A	3,10	0,50	0,80	0,20	4,60
	Type B	15,40	2,50	18,50	1,20	37,60
	Kentucky reference snuff 1S3	12,60	2,20	5,40	0,60	20,80
Sweden**		3,80	1,30	-	-	8,80
Sudan**		188-7870	8-102	-	-	141-3080
US *	Copanhagen Skoal, Original	8.7±1.4	1.9±0.6	-	-	17.2±3.0
	Fine cut Skoal, Bandits	8.2±1.3	1.3±0.1	-	-	14.9±2.5
	Straight Kodiak	5.1±1.0	0.9±0.3	-	-	8.2±1.7
	Wintergreen Hawken	6.3±1.1	0.6±0.2	-	-	11.0 ±2.4
	Wintergreen	3.1±0.3	0.2±0.04	-	-	4.1±0.4

Abbreviations: DM: Dry matter, Mp: Maras Powder, NNN: nitrosonornicotine, NNK: 4-(nitrosometil-amino)-1-(3-pyridil)-butanon, NAT: N' Nitrosoanatabine, NAB: N' Nitrosoanabasine, total TSNA: Tobacco specific nitrosamines
*In Reference 1.
**In Reference 21.

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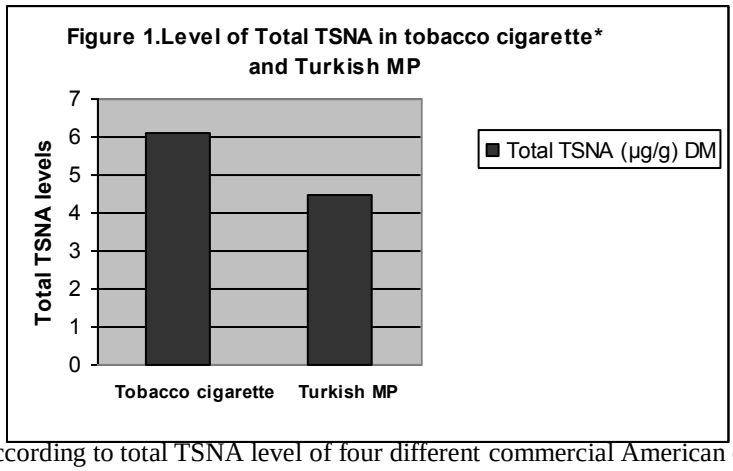

*: Calculated mean according to total TSNA level of four different commercial American cigarette brands [from Stepanov 2002)].